

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ :	A1	(11) International Publication Number: WO 95/05814 (43) International Publication Date: 2 March 1995 (02.03.95)
A61K 31/28, 33/24		
(21) International Application Number:	PCT/GB94/01817	(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FL, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD).
(22) International Filing Date:	19 August 1994 (19.08.94)	
(30) Priority Data:	9317686.5 25 August 1993 (25.08.93) GB	Published <i>With international search report.</i>
(71) Applicant (<i>for all designated States except US</i>):	JOHNSON MATTHEY PUBLIC LIMITED COMPANY [GB/GB]; 78 Hatton Garden, London EC1N 8JP (GB).	
(72) Inventors; and		
(75) Inventors/Applicants (<i>for US only</i>):	ABRAMS, Michael, Jeffrey [US/US]; Creek Road, RD No. 2, Box 31, Glenmore, PA 19343 (US). FRICKER, Simon, Paul [GB/GB]; 30 Glebe Road, Sonning-on-Thames, Reading RG4 0XH (GB). MURRER, Barry, Anthony [GB/GB]; 17 Carnarvon Road, Reading RG1 5SB (GB). VAUGHAN, Owen, John [GB/SE]; Folvagen 14, S-152 57 Ostertalje (SE).	
(74) Agent:	WISHART, Ian, Carmichael; Johnson Matthey Technology Centre, Blounts Court, Sonning Common, Reading RG4 9NH (GB).	

(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING METAL COMPLEXES

(57) Abstract

New pharmaceutical compositions and pharmaceutical compositions comprising metal complexes have activity against diseases caused by or related to overproduction or localised high concentration of nitric oxide in the body.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

- 1 -

PHARMACEUTICAL COMPOSITIONS COMPRISING METAL COMPLEXES

This invention relates to new pharmaceutical compositions and to pharmaceutical compositions having activity against diseases caused by or related 5 to overproduction or localised high concentration of nitric oxide in the body.

Nitric oxide (NO) plays a varied and vital role in the human body. For example, NO plays a vital role in the control of blood pressure; it acts as a neurotransmitter; it plays a role in inhibition of platelet aggregation (important in 10 thrombosis or blockages of the blood vessels), and in cytostasis (important in

- 2 -

fighting of tumours). Overproduction of NO however, has been implicated in a number of disease states, including vascular/pressor diseases such as septic shock, post-ischaemic cerebral damage, migraine, and dialysis induced renal hypotension; immunopathologic diseases such as hepatic damage in inflammation and sepsis, 5 allograft rejection, graft versus host diseases, diabetes and wound healing; neurodegenerative diseases such as cerebral ischaemia, trauma, chronic epilepsy, Alzheimer's disease, Huntington's disease, and AIDS dementia complex; and side effects of treatment such as restenosis following angioplastic treatment and secondary hypotension following cytokine therapy.

10

Pharmacological modulation of nitric oxide in any of these disease states should prove extremely beneficial.

One above-mentioned disease relating to overproduction of NO, is 15 septic shock. This is precipitated by local septicaemia or endotoxaemia, (high local levels of bacterial endotoxins). The result is activation of macrophages, lymphocytes, endothelial cells and other cell types capable of producing NO, further mediated by cytokine production by these cells. The activated macrophages produce excess NO which causes vasodilation of the blood vessels, and results in 20 local vascular damage and vascular collapse. This destruction of vascular integrity may be so great that it leads to the collapse of haemodynamic homeostasis, the end result being death.

- 3 -

Current ideas for pharmacological modulation of nitric oxide in such diseases are based on dealing with the mediators of septic shock, such as cytokines, endotoxins, and platelet activating factor (PAF). The approaches include use of antibodies to cytokines such as tumour necrosis factor (TNF), receptor antagonists 5 such as interleukin 1, (IL-1), antibodies to lipopolysaccharide (the endotoxin produced by gram negative bacteria, and PAF antagonists. All such approaches while challenging a factor mediating septic shock, do not attempt to deal with the aetiology, or cause, of the disease. Recent advances in understanding of NO have lead to the proposal that inhibitors of the NO synthase enzyme, such as 10 N^{G} -monomethyl-L-arginine (L-NMMA), may be useful in the treatment of septic shock and other NO overproduction related to diseases since they inhibit NO production. While these inhibitors have shown some utility in animal models and preliminary clinical studies, they have the disadvantage of undesirably inhibiting total NO synthesis in the body.

15

An aim of the present invention is to provide new and previously indicated pharmaceutical compositions which are able to modulate NO levels in the body by scavenging, or removing, NO in situ so that necessary NO synthesis continues while dangerous excesses are removed. We have found that certain metal complexes have the ability to carry out this important role.

Some metal complexes are known in pharmaceutical compositions for the treatment of diseases of the human body. For example, certain complexes of platinum and ruthenium have been used or indicated in the treatment of cancer.

- 4 -

Metal complexes have not however been previously indicated in the treatment of NO overproduction related diseases.

This invention provides for the use of a neutral, anionic or cationic
5 metal complex having at least one site for coordination with NO, of formula



in the manufacture of a medicament for the attenuation of NO levels where NO is
10 implicated in disease,

where: M is a metal ion or a mixture of metal ions;
 X is a cation or a mixture of cations;
 L is a ligand, or mixture of ligands each containing at least two
15 different donor atoms selected from the elements of Group IV,
 Group V or Group VI of the Periodic Table;
 Y is a ligand, or a mixture of the same or different ligands each
 containing at least one donor atom or more than one donor
 atom, which donor atom is selected from the elements of
20 Group IV, Group V or Group VI of the Periodic Table;
and Z is a halide or pseudohalide ion or a mixture of halide ions and
 pseudohalide ions;
a = 1-3; b = 0-12; c = 0-18; d = 0-18; e = 0-18; and n = 0-10;

- 5 -

provided that at least one of c, d and e is 1 or more;

and where c is 0; b is also 0;

and where a is 1; c, d and e are not greater than 9;

5 and where a is 2; c, d and e are not greater than 12.

By "complex" in this specification is meant a neutral complex or anionic or cationic species.

10 The term "Group" which is used herein is to be understood as a vertical column of the periodic table in which elements of each Group have similar physical and chemical properties. The definition of the Periodic Table is that credited to Mendeleev; Chambers Dictionary of Science and Technology, 1974.

Published by W & R Chambers Ltd.

15

This invention may also be stated as providing a method of attenuation of NO levels where NO is implicated in diseases of the human body, comprising administering a pharmaceutical composition containing a neutral, anionic or cationic metal complex of formula I.

20

This invention may also provide for the use of a neutral, anionic or cationic metal complex of formula I in the manufacture of a medicament for the treatment of NO overproduction related disease.

- 6 -

This invention may also be stated as providing a method of treatment of diseases of the human body resultant of overproduction of NO in the human body, comprising administering a pharmaceutical composition containing a neutral, anionic or cationic metal complex of formula I.

5

Where the formula I represents an anionic species, a cation will also be present. Where formula I represents a cationic species, an anion will also be present. The metal complexes may be hydrated.

10

Preferably, M is a first, second or third row transition metal ion. For example, M may be an Rh, Ru, Os, Mn, Co, Cr or Re ion, and is preferably an Rh, Ru or Os ion.

15

Suitably M is in an oxidation state III. We have found surprisingly that when the metal ion for example ruthenium is in oxidation state III, the rate at which it binds with NO is significantly faster than when it is in oxidation state II.

20

X may be any cation, such as a mono-, di- or tri-valent cation. Suitable cations may be H⁺, K⁺, Na⁺, NH₄⁺ or Ca²⁺. Conveniently X may be H⁺, K⁺ or Na⁺.

Preferably, L is a ligand containing both nitrogen and oxygen donor atoms. Examples of suitable such ligands include ethylenediamine -N,N'-diacetic acid (edda), ethylenediaminetetraacetic acid (edta), nitrilotriacetic acid (nta),

- 7 -

dipicolinic acid (dipic), picolinic acid (pic), diethylenetriaminepentaacetic acid (dtpa), thiobis(ethylenenitrilo)tetraacetic acid (tedta), dithioethanebis(ethylene-nitrilo)tetraacetic acid (dtedta) and N-(2-hydroxyethyl)ethylenediamine-triacetic acid (hedtra).

5

Preferably, Y is a ligand containing nitrogen, oxygen, sulphur, carbon, or phosphorus donor groups. Suitable nitrogen donor groups may be for example ammine, amine, nitrile and nitride or derivations thereof. Suitable oxygen donor groups may be for example carboxylic acid, ester or derivations thereof, 10 water, oxide, sulphoxide, hydroxide, acetate, lactate, propionate, oxalate and maltolate. Suitable sulphur donor groups may be for example sulphoxide, dialkylsulphide, dithiocarbamate or dithiophosphate. Suitable carbon donor groups may be for example carbon monoxide or isocyanide. Suitable phosphorus donor groups may be for example trialkylphosphine.

15

Z may be any halide and is preferably chloride, bromide or iodide. Most conveniently, Z is chloride.

20

Examples of metal complexes for use according to the present invention include optionally hydrated ruthenium complexes of formula



- 8 -

where L" is an amide or ester or derivative thereof, or a polydentate aminocarboxylate ligand, for example edta, nta, dipic, pic, edda, tropolone, dtpa, hedtra, teda or dteda or diamide of edta or dtpa or a mixture of any of these, and Y is as defined above and may for example be selected from acetylacetone (acac),
5 a β -diketonate, water, dimethylsulphoxide (dmso), carboxylate, bidentate carboxylate, catechol, kojic acid, maltol, hydroxide, tropolone, malonic acid, oxalic acid, 2,3-dihydroxynaphthalene, squaric acid, acetate, a sulphate and a glycolate. The skilled addressee will be able to substitute other known ligands at Y and which will fall within the scope of the inventions. Preparative methods of teda, dteda
10 and diamide of edta and dtpa are described in the following references respectively:

P Tse & JE Powell, Inorg Chem, (1985), 24, 2727

G Schwartzenbach, H Senner, G Anderegg, Helv Chim Acta 1957,
15 40, 1886

MS Konings, WC Dow, DB Love, KN Raymond, SC Quay and
SM Rocklage, Inorg Chem (1990), 29, 1488-1491

20 PN Turowski, SJ Rodgers, RC Scarro and KN Raymond, Inorg Chem (1988), 27, 474-481.

Where the complex of formula II is an anion, a cation will be required. For example the complexes of formula II are present in

- 9 -

K[Ru(Hedta)Cl]2H₂O

[Ru(H₂edta)(acac)]

K[Ru(hedtra)Cl]H₂O

K[Ru(dipic)₂]H₂O

5 (H₂pic)[RuCl₂(pic)₂](Hpic)H₂O

K[Ru(H₂edta)Cl₂]H₂O

K[Ru(Hnta)₂]½H₂O

K[Ru(H₂dtpa)Cl]H₂O

[Ru(Hhedtra)acac]H₂O

10 [Ru(Hhedtra)trop]

[Ru(H₃dtpa)Cl]

Complexes of formula II have not to the best of our knowledge been previously indicated in any pharmaceutical composition. Therefore the present invention also provides a pharmaceutical composition containing an optionally hydrated ruthenium complex of formula II.

Further examples of metal complexes for use according to the present invention include optionally hydrated complexes of formula III

20

[M₁₋₃Y₁₋₁₈Cl₀₋₁₈]^{(0-6)±}

formula III

Where Y is a sulphur donor ligand. For example, such complex is present in

- 10 -



5 Complexes of formula III in which Y is a sulphur donor ligand have
not to the best of our knowledge been previously indicated in any pharmaceutical
composition. Therefore, the present invention also provides a pharmaceutical
composition containing an optionally hydrated complex of formula III wherein Y
is a sulphur donor ligand.

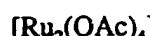
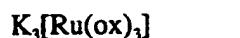
10 Yet further examples of metal complexes for use according to the
present invention include optionally hydrated complexes of ruthenium of formula



where M''' is ruthenium and Y''' is an oxygen-donor ligand such as acetate, lactate, water, oxide, propionate (COEt), oxalate (ox), or maltolat (maltol) or a combination of these. For example complexes of formula III are present in



20 [Ru₃O(lac)₆](lac)



- 11 -

Some complexes of formula III have not to the best of our knowledge been previously indicated in any pharmaceutical composition. Therefore the present invention also provides a pharmaceutical composition containing an optionally hydrated complex of ruthenium of formula III wherein M''' is ruthenium and Y''' is an oxygen-donor ligand selected from the group acetate, lactate, oxide, propionate and maltolate.

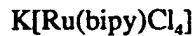
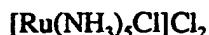
10



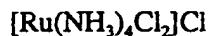
formula IV

where Y^{IV} is a nitrogen-donor ligand such as ammine, ethylenediamine (en), pyridine (py), 1,10-phenanthroline (phen), 2,2'-bipyridine (bipy) or 1,4,8,11-tetra-15 azacyclotetradecane (cyclam), 2,3,7,8,12,13,17,18-octaethylporphyrin (oep) or a combination of these. For example complexes of formula IV are present in

20



- 12 -



Some complexes of formula IV have not to the best of our
 5 knowledge been previously indicated in any pharmaceutical composition. Therefore the present invention also provides a pharmaceutical composition containing an optionally hydrated complex of ruthenium of formula IV wherein Y^{IV} is a nitrogen-donor ligand selected from the group en, py, phen, bipy, cyclam and oep. Derivations of these ligands can be prepared by a skilled addressee and which will
 10 fall within the scope of the inventions.

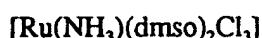
Still further examples of metal complexes for use according to the present invention include optionally hydrated complexes of ruthenium or osmium of general formula

15

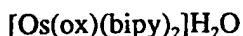


formula V

where Y^{V} is a combination of donor ligands such as are described hereinabove, for example ammine, dmso, oxalate, bipy, acac and MeCN. Complexes of formula V
 20 are present in for example



- 13 -



5 The complex ions of the latter two compounds above have not to
the best of our knowledge been previously indicated in any pharmaceutical
composition. Therefore the present invention also provides a pharmaceutical
composition containing an optionally hydrated complex of formula $[\text{Os}(\text{ox})(\text{bipy})_2]$;
and further a pharmaceutical composition containing an optionally hydrated
10 complex of formula $[\text{Ru}(\text{acac})_2(\text{MeCN})_2]^+$.

In use the complexes of the present invention may be included as
an active component in a pharmaceutical composition containing an optionally
hydrated complex of any of formulae I-V, in admixture with a pharmaceutically
15 acceptable carrier or diluent. Said pharmaceutical composition may be formulated
according to well known principles, and may be in the form of a solution or
suspension for parenteral administration in single or repeat doses or be in capsule,
tablet, dragee, or other solid composition or as a solution or suspension for oral
administration, or formulated into pessaries or suppositories, or sustained release
20 forms of any of the above. The solution or suspension may be administered by a
single or repeat bolus injection or continuous infusion, or any other desired
schedule. Suitable diluents, carriers, excipients and other components are known.
Said pharmaceutical composition may contain dosages determined in accordance
with conventional pharmacological methods, suitable to provide active complexes

- 14 -

in the dosage range in humans of 1mg to 10g per day. Actual required dosage is largely dependent on where in the body there is the excess concentration of NO and for how long overproduction continues or attenuation of NO levels, where NO is implicated in disease, is required.

5

This invention will now be illustrated by Example.

A number of commercially available compounds, and compounds prepared by routes known in the literature, containing the complexes of the present 10 invention were tested *in vitro*, *in vitro* cell culture, and *ex-vivo* in order to determine ability to coordinate with NO. The complexes tested were as follows:

<u>Example</u>	<u>Compound</u>	<u>Literature Reference for Preparation</u>
15 1	K[Ru(Hedta)Cl]2H ₂ O	AA Diamantis & JV Dubrawski, Inorg.Chem.,(1981), <u>20</u> ,1142-50
20 2	[Ru(H ₂ edta)(acac)]	AA Diamantis & JV Dubrawski, Inorg.Chem.,(1983), <u>22</u> ,1934-36
25 3	K[Ru(hedtra)Cl]H ₂ O	HC Bajaj & R van Eldik, Inorg.Chem.(1982), <u>28</u> ,1980-3
4	K[Ru(dipic) ₂]H ₂ O	NH Williams & JK Yandell, Aust.J.Chem.(1983), <u>36</u> (12),2377-2386
5.	(H ₂ pic)[RuCl ₂ (pic) ₂](Hpic)H ₂ O	JD Gilbert, D Rose & G Wilkinson, J.Chem.Soc.(A),(1970),2765-9

- 15 -

- | | | |
|----|--|--|
| 6 | K[Ru(H ₂ edta)Cl ₂]H ₂ O | AA Diamantis & JV Dubrawski,
Inorg.Chem.(1981), <u>20</u> ,1142-50 |
| 7 | K[Ru(H ₂ nta) ₂]½H ₂ O | MM Taqui Khan, A Kumar &
Z Shirin,
J. Chem. Research (M), (1986),1001-
1009 |
| 8 | K[Ru(H ₂ dtpa)Cl]H ₂ O | MM Taqui Khan, A Kumar &
Z Shirin,
J. Chem. Research (M). (1986); 1001-
1009 |
| 9 | [Ru ₃ O(lac) ₆](lac) | A Spencer & G Wilkinson,
J. Chem. Soc. Dalton Trans, (1972),
1570-77 |
| 10 | [Ru ₃ O(OAc) ₆](OAc) | A Spencer & G Wilkinson,
J.Chem. Soc. Dalton Trans. (1972),
1570-77 |
| 11 | [Ru ₂ (OAc) ₄]NO ₃ | M Mukaida, T Nomura & T Ishimori,
Bull. Chem. Soc. Japan, (1972) <u>45</u> ,
2143-7 |
| 12 | [Ru ₂ (OCOEt) ₄]NO ₃ | A Bino, FA Cotton & TR Felthouse,
Inorg. Chem. (1979), <u>18</u> , 2599-2604 |
| 30 | 13 K ₃ [Ru(ox) ₃] | CM Che, SS Kwong, CK Poon,
TF Lai & TCW Mak
Inorg. Chem. (1985), <u>24</u> , 1359-63 |

- 16 -

- | | | |
|----|---|--|
| 14 | [Ru ₂ (OAc) ₄]Cl | RW Mitchell, A Spencer &
G Wilkinson
J. Chem. Soc. Dalton Trans., (1973),
846-54 |
| 5 | | |
| 15 | [Ru(NH ₃) ₅ Cl]Cl ₂ | AD Allen, F Bottomley, RO Harris,
VP Reinsalu & CV Senoff
J. Amer. Chem. Soc. (1967), <u>89</u> ,
5595-5599 |
| 10 | | |
| 16 | [Ru(en) ₃]I ₃ | TJ Meyer & H Taube
Inorg. Chem. (1968), <u>7</u> , 2369-2379 |
| 15 | 17 K[RuCl ₄ (phen)]H ₂ O | BR James & RS McMillan
Inorg. Nucl. Chem. Lett. (1975),
<u>11(12)</u> 837-9 |
| 20 | 18 [Ru(cyclam)Cl ₂]Cl | PK Chan, DA Isabirye & CK Poon
Inorg. Chem. (1975), <u>14</u> , 2579-80 |
| 19 | K[RuCl ₄ (bipy)] | BR James & RS McMillan
Inorg. Nucl. Chem. Lett. (1975),
<u>11(12)</u> , 837-9 |
| 25 | 20 [RuCl ₃ (dmso) ₂ (NH ₃)] | Patent: International Publication No
WO 91/13553 |
| 30 | 21 [Ru(NH ₃) ₆]Cl ₃ | Matthey Catalogue Sales: Cat No
[190245] |

- 17 -

- | | | |
|----|--|--|
| 22 | cis-[RuCl ₂ (dmso) ₄] | EA Alessio, G Mestroni, G Nardin,
WM Attia, M Calligaris, G Sava &
S Zorget
<i>Inorg. Chem.</i> (1988), <u>27</u> , 4099-4106 |
| 5 | | |
| 23 | cis-[RuCl ₂ (dmso) ₃ (NH ₃)] | M Henn, E Alessio, G Mestroni,
M Calligaris & WM Attia
<i>Inorg. Chim. Acta</i> , (1991), <u>187</u> , 39-50 |
| 10 | 24 [RuCl ₃ (dmso) ₃] | E Alessio, G Balducci, M Calligaris,
G Costa, WM Attia & G Mestroni
<i>Inorg. Chem.</i> (1991), <u>30</u> , 609-618 |
| 15 | 25 [Ru(mtc) ₃] | AR Hendrickson, JM Hope &
RL Martin
<i>J. Chem. Soc. Dalton Trans.</i> (1976),
<u>20</u> , 2032-9 |
| 20 | 26 [Ru(maltol) ₃] | WP Griffith & SJ Greaves
<i>Polyhedron</i> , (1988), <u>7(19)</u> , 1973-9 |
| 25 | 27 [Ru(acac) ₂ (MeCN) ₂]CF ₃ SO ₃ | Y Kasahara, T Hoshino, K Shimizu &
GP Sato
<i>Chem. Lett.</i> (1990), <u>3</u> , 381-4 |
| | 28 K ₂ [RuCl ₅ (H ₂ O)] | Matthey Catalogue Sales: Cat No
[190094] |

- 18 -

29	[Os(ox)(bipy) ₂] _. H ₂ O	DA Buckingham, FP Dwyer, HA Goodwin & AM Sargeson Aust.J.Chem.(1964),325-336
5		GM Bryant, JE Fergusson & HKJ Powell Aust.J.Chem.(1971), <u>24</u> (2),257-73
10	30 [Ru(NH ₃) ₄ Cl ₂]Cl	SD Pell, MM Sherban, V Tramintano & MJ Clarke Inorg Synth, (1989), <u>26</u> , 65.
15	31 [Ru(Hedtra)(dppm)]	MM Taqui Khan, K Venkatasubramanian, Z Shirin, MM Bhadbhade J Chem Soc Dalt Trans (1992), 885-890
20	32 Ru(oep)Ph	M Ke, SJ Rettig, BR James and D Dolphin J Chem Soc Chem Commun (1987), 1110

A number of new compounds were prepared according to the following protocols. The first four compounds are examples of ruthenium complexes of formula [Ru(H_{0.6}L'')_{1.3} Y_{0.2} Cl_{0.4}]^{(0-4)±} (formula II), the subsequent 25 two are examples of [M_{1.3}Y_{1.8}Cl_{0.18}]^{(0-6)±} (formula III).

- 19 -

Preparation of [Ru(Hhedtra)acac].H₂O

Excess acetylacetone (1cm³) was added to an aqueous solution (5cm³) of K[Ru(hedtra)Cl] (0.5g). The solution colour changed to violet. The mixture was warmed for 20 minutes then left to stand at room temperature for 20 minutes. The violet solution was extracted with chloroform (20cm³). The extraction was repeated twice more. A violet product precipitated from the aqueous fraction. The product was filtered, washed in acetone and dried in vacuo, yield 0.1g (18%).

Anal. Calc. for C₁₅H₂₅O₁₀N₂Ru: C, 36.43; H, 5.11; N, 5.70. Found: C, 36.16; H, 5.42; N, 5.61%.

Preparation of [Ru(Hhedtra)trop]2H₂O

A three-fold excess of tropolone (0.78g) dissolved in 50:50 water/absolute ethanol (5cm³) was added to a warm aqueous solution of K[Ru(hedtra)Cl] (10cm³). The mixture was heated for 1 hour. On cooling, the dark green mixture was extracted with 3 x 20cm³ portions of dichloromethane. On standing, a dark green product precipitated from the aqueous fraction. The product was filtered, washed with water (1cm³), ether and dried in vacuo, yield 0.4g (36%).

Anal. Calc. for C₁₇H₂₂N₂O₉Ru.2H₂O: C, 38.13; H, 4.86; N, 5.23. Found: C, 38.55; H, 4.67; N, 5.28%.

- 20 -

Preparation of [Ru(H₃dtpa)Cl]

K₂[RuCl₅H₂O].xH₂O (1g) was suspended in HClO₄ (15cm³, 1mM) and diethylenetriaminepentaacetic acid (1.05g) added. The reaction mixture was 5 heated under reflux for 1.5 hours forming a yellow/brown solution. On cooling a yellow product crystallised which was collected by filtration, washed with 90% absolute ethanol/water, diethyl ether and dried in vacuo, yield 0.75g, 53%. Anal. calcd. for C₁₄H₂₁N₃O₁₀ClRu: C, 31.85; H, 3.98; N, 7.96; Cl, 6.73. Found: C, 29.77; H, 3.81; N, 7.36; Cl, 6.64.

10

Preparation of K[RuHHBEDCl]3H₂O

0.41g of K₂[RuCl₅]xH₂O was dissolved in water (20ml). To this solution was added 1 equivalent (0.39g) of N,N'di(2-hydroxy-benzyl)ethylene-15 diamine N,N-diacetic acid (hbcd) dissolved in water (50ml) with KOH (0.12g) and MeOH (1ml). This mixture was heated at reflux for 90 minutes. Upon cooling a dark, insoluble precipitate formed. This material was removed by filtration and the resulting red-violet solution was taken to dryness by rotary evaporation. Trituration with water and washing with acetone yielded 90mg of a dark solid.
20 Anal. calcd. for C₁₈H₂₂N₂O₉RuClK: C, 36.89; H, 3.96; N, 4.78; Cl, 6.04. Found: C, 37.09; H, 4.23; N, 4.92; Cl, 6.28.

- 21 -

Preparation of Ru(S₂CNCH₂CH₂NMeCH₂CH₂)₃½H₂O

Me₄N[S₂CNCH₂CH₂NMeCH₂CH₂] was made by the standard method and crystallised from methanol-ether in 71% yield.

5 RuCl₃·xH₂O, 0.50g, 2.15mmol was refluxed in 30ml of methanol for 10 minutes and cooled. 1.87g, 7.50mmol of Me₄N[S₂CNCH₂CH₂NMeCH₂CH₂] was added and the mixture refluxed for 16 hours. After cooling 0.72g of crude product was filtered off, dissolved in dichloromethane and filtered. The filtrate was loaded into 15cc of basic alumina and eluted with dichloromethane. Removal of solvent 10 and crystallisation from dichloromethane with ether by vapour-phase diffusion gave 0.51g, 0.80mmol, 37% of brown-black crystals, Ru(S₂CNCH₂CH₂NMeCH₂CH₂)₃½H₂O.

Analysis for C₁₈H₃₄N₆O₅RuS₆: Calc: C, 34.00; H, 5.39; N, 13.22; S, 30.25. Found: C, 34.21; H, 5.47; N, 13.12; S, 30.36.

15

Preparation of Ru[S₂P(OC₂H₄OC₂H₄OMe)₂]₃

K[S₂P(OC₂H₄OC₂H₄OMe)₂]₃ was made by standard method and crystallised from methanol in 76% yield.

20

RuCl₃·xH₂O, 1.00g, 4.30mmol was refluxed in 50ml of 0.1 N HCl with 1ml of ethanol for 20 minutes and cooled. To this solution was added 5.28g (excess) K[S₂P(OC₂H₄OC₂H₄OMe)₂] and the mixture stirred at 30°C for 1 hour. the reaction mixture was extracted with dichloromethane and the solvent removed.

- 22 -

The residue was extracted with ether-hexane and solvents removed. This residue was crystallised from 25ml of hot ether by cooling to -20°C giving 2.98g of red crystals. 2.41g of the crude product was purified by chromatography on 60cc of silica gel with 5% ethanol in ether. The first band was collected, reduced to dryness and crystallised from ether by cooling to -20°C. The yield of red crystals, Ru(S₂P{OC₂H₄OC₂H₄OMe})₂, was 2.16g, 56%.

Analysis for C₃₀H₆₆O₁₈P₃RuS₆: Calc: C, 32.72; H, 6.04; S, 17.47. Found: C, 32.68; H, 6.08; S, 17.16.

10 In the *in vitro* tests, which were carried out in an atmosphere of argon, each compound (1 x 10⁴ moles) was dissolved in double-distilled deionized and deoxygenated water. The resulting solution was placed in a three-necked pear-shaped flask and stirred by a magnetic stirrer at constant speed of 1000rpm, at a constant temperature in the range 20°C-24°C. A manometer was attached to the
15 flask, and purified, dried nitric oxide gas (known volume in the range 3-5cm³) was introduced via a septum, using a gas syringe, at atmospheric pressure into the headspace above the reaction solution. The pressure within the flask was recorded periodically over a period of one hour.

20 A control experiment was carried out according to the above but without any complex present.

- 23 -

The recorded pressures in association with the results of the control experiment were analysed in order to determine the rate of NO uptake as a function of time for each compound tested.

5 On completion of each *in vitro* test, the reaction solution was freeze-dried. An infrared spectrum of the freeze-dried product provided information on metal-NO bond formation.

10 In the *in vitro* cell culture tests, murine (RAW264) macrophage cell lines, which can be induced to produce nitric oxide, were seeded, 10^6 cells/well, onto 24 well culture plates of 2ml volume per well, in Eagles modified minimal essential medium (MEM) plus 10% foetal bovine serum without phenol red.

15 The cells were activated to produce nitric oxide, with 10 μ g/ml lipopolysaccharide and 100 units/ml interferon γ for 18 hours. Concurrently, test compounds made up in MEM were added at non-cytotoxic concentrations.

20 Control cells as above, which were activated to produce nitric oxide as above, but to which no test compound was added, were used as a measure of the amount of nitric oxide produced by the cells during the tests.

Background nitric oxide was assessed by measurement of nitrate and nitrite in cells which were not activated.

- 24 -

Cell viability was confirmed by Trypan blue dye exclusion at the end of the incubation period.

Nitric oxide was determined by measurement of nitrate and nitrite in
5 the cell supernatant. These anions are the stable end-products of reactions of NO in solution. Such reactions may or may not be catalysed in biological systems. The sum of nitrite and nitrate concentrations gives the total NO production. Nitrite was determined using the Griess reaction in which nitrite reacts with 1% sulphanilamide in 5% H₃PO₄/0.1% naphthylethylenediamine dihydrochloride to
10 form a chromophore absorbing at 540nm. Nitrate was determined by reducing nitrate to nitrite with a bacterial nitrate reductase from *Pseudomonas oleovorans* and then measuring nitrite with the Griess reaction. In the absence of test compounds nitrite concentration plus nitrate concentration is equal to total nitric oxide production. The effect of test compounds on available nitric oxide (measured
15 as nitrite + nitrate) was determined. The reduction in available nitric oxide compared with the control level may be taken as an indication of the degree of binding of NO by the test compounds.

In the *ex vivo* tests, segments of rat tail artery (0.8-1.5cm) were
20 dissected free from normotensive adult Wistar rats. The arteries were internally perfused with Krebs solution (mM: NaCl 118, KCl 4.7, NaHCO₃, 25, NaH₂PO₄ 1.15, CaCl₂ 2.5, MgCl₂ 1.1, glucose 5.6 and gassed with 95% O₂/5% CO₂ to maintain a pH of 7.4) in a constant flow perfusion apparatus. A differential pressure transducer located upstream of the vessel detected changes in back

- 25 -

pressure. The rat tail artery preparation was pre-contracted with 6.5 μ M phenylephrine to give a physiologically normal pressure of 100-120mm Hg. The pre-contracted vessels were then perfused with the test compound. The arteries were perfused with Krebs solution between applications of test compound to wash 5 out the test compound.

Pressure changes in the system served to indicate artery vasoconstriction. The vasoconstriction is a direct result of the removal of endogenous nitric oxide (edrf) from the endothelial cells of the rat tail artery.

10

RESULTS

The results of the *in vitro*, *in vitro* cell culture and *ex-vivo* tests were as follows:

15

IN VITRO TESTS

EXAMPLE 1 : K[Ru(Hedta)Cl]2H₂O

20

A pressure decrease indicated binding of NO to the metal compound.

This is illustrated in Figure 1.

The IR spectrum showed a peak at 1897cm⁻¹, indicating the presence of a Ru-NO bond.

- 26 -

EXAMPLE 2 : [Ru(H₂edta)(acac)]

The IR spectrum showed a peak at 1896cm⁻¹, indicating the presence
of a Ru-NO bond.

5

EXAMPLE 3 : K[Ru(hedtra)Cl]H₂O

A pressure decrease indicated binding of NO to the metal compound.
This is illustrated in Figure 1.

10

The IR spectrum showed a peak at 1889cm⁻¹, indicating the presence
of a Ru-NO bond.

EXAMPLE 4 : K[Ru(dipic)₂]H₂O

15

A pressure decrease indicated binding of NO to the metal compound.
This is illustrated in Figure 1.

20

of a Ru-NO bond.

The IR spectrum showed a peak at 1915cm⁻¹, indicating the presence
of a Ru-NO bond.

- 27 -

EXAMPLE 5 : $(H_2pic)[RuCl_2(pic)_2](Hpic)H_2O$

The IR spectrum showed a peak at 1888cm^{-1} , indicating the presence of a Ru-NO bond.

5

EXAMPLE 6 : $K[Ru(H_2edta)Cl_2]H_2O$

A pressure decrease indicated binding of NO to the metal compound. This is illustrated in Figure 1.

10

The IR spectrum showed a peak at 1896cm^{-1} , indicating the presence of a Ru-NO bond.

EXAMPLE 7 : $K[Ru(Hnta)_2]\frac{1}{2}H_2O$

15

A pressure decrease indicated binding of NO to the metal compound. This is illustrated in Figure 1.

20

The IR spectrum showed a peak at 1889cm^{-1} , indicating the presence of a Ru-NO bond.

- 28 -

EXAMPLE 8 : K[Ru(H₂dtpa)Cl]H₂O

A pressure decrease indicated binding of NO to the metal compound.

This is illustrated in Figure 1.

5

The IR spectrum showed a peak at 1905cm⁻¹, indicating the presence of a Ru-NO bond.

EXAMPLE 9 : [Ru₃O(lac)₆](lac)

10

The IR spectrum showed a peak at 1884cm⁻¹, indicating the presence of a Ru-NO bond.

EXAMPLE 10 : [Ru₃O(OAc)₆](OAc)

15

The IR spectrum showed a peak at 1877cm⁻¹, indicating the presence of a Ru-NO bond.

EXAMPLE 11 : [Ru₂(OAc)₄]NO₃

20

The IR spectrum showed a peak at 1891cm⁻¹, indicating the presence of a Ru-NO bond.

- 29 -

EXAMPLE 12 : [Ru(OCOEt)₄]NO₃

The IR spectrum showed a peak at 1891cm⁻¹, indicating the presence of a Ru-NO bond.

5

EXAMPLE 13 : K₃[Ru(ox)₃]

The IR spectrum showed a peak at 1889cm⁻¹, indicating the presence of a Ru-NO bond.

10

EXAMPLE 14 : [Ru₂(OAc)₄]Cl

The IR spectrum showed a peak at 1895cm⁻¹, indicating the presence of a Ru-NO bond.

15

EXAMPLE 15 : [Ru(NH₃)₅Cl]Cl₂

The IR spectrum showed two peaks at 1909cm⁻¹ and 1928cm⁻¹, indicating the presence of a Ru-NO bond.

20

EXAMPLE 16 : [Ru(en)₃]I₃

The IR spectrum showed a peak at 1906cm⁻¹, indicating the presence of a Ru-NO bond.

- 30 -

EXAMPLE 17 : K[RuCl₄(phen)]H₂O

The IR spectrum showed a peak at 1904cm⁻¹, indicating the presence
of a Ru-NO bond.

5

EXAMPLE 18 : [Ru(cyclam)Cl₂]Cl

The IR spectrum showed a peak at 1895cm⁻¹, indicating the presence
of a Ru-NO bond.

10

EXAMPLE 19 : K[RuCl₄(bipy)]

The IR spectrum showed a peak at 1885cm⁻¹, indicating the presence
of a Ru-NO bond.

15

EXAMPLE 20 : [RuCl₃(dmso)₂(NH₃)]

The IR spectrum showed a peak at 1889cm⁻¹, indicating the presence
of a Ru-NO bond.

20

EXAMPLE 21 : [Ru(NH₃)₆]Cl₃

The IR spectrum showed a peak at 1910cm⁻¹, indicating the presence
of a Ru-NO bond.

- 31 -

EXAMPLE 22 : cis-[RuCl₂(dmso)₄]

The IR spectrum showed a peak at 1881cm⁻¹, indicating the presence of a Ru-NO bond.

5

EXAMPLE 23 : cis-[RuCl₂(dmso)₃(NH₃)]

The IR spectrum showed a peak at 1893cm⁻¹, indicating the presence of a Ru-NO bond.

10

EXAMPLE 24 : [RuCl₃(dmso)₃]

The IR spectrum showed a peak at 1880cm⁻¹, indicating the presence of a Ru-NO bond.

15

EXAMPLE 25 : [Ru(mtc)₃]

The IR spectrum showed a peak at 1862cm⁻¹, indicating the presence of a Ru-NO bond.

20

EXAMPLE 26 : [Ru(maltol)₃]

The IR spectrum showed a peak at 1866cm⁻¹, indicating the presence of a Ru-NO bond.

- 32 -

EXAMPLE 27 : [Ru(acac)₂(MeCN)₂]CF₃SO₃

The IR spectrum showed a peak at 1899cm⁻¹, indicating the presence of a Ru-NO bond.

5

EXAMPLE 28 : K₂[RuCl₅(H₂O)]

The IR spectrum showed a peak at 1903cm⁻¹, indicating the presence of a Ru-NO bond.

10

EXAMPLE 29 : [Os(ox)(bipy)₂]H₂O

The IR spectrum showed a peak at 1894cm⁻¹, indicating the presence of a Os-NO bond.

15

IN VITRO CELL CULTURE TESTS

Results are shown in Table 1 and Figure 2.

20

EXAMPLE 1: K[Ru(Hedta)Cl]2H₂O

Available nitric oxide was reduced in a dose-dependent fashion with a maximum reduction of 75% at a concentration of 100μM.

- 33 -

EXAMPLE 2: [Ru(H₂edta)(acac)]

Available nitric oxide was reduced by 82% at 100µM test compound.

5 EXAMPLE 3: K[Ru(Hedtra)Cl]H₂O

Available nitric oxide was reduced by 42% at 100µM.

10 EXAMPLE 6: K[Ru(H₂edta)Cl₂]H₂O

Available nitric oxide was reduced by 77% at 100µM test compound.

15 EXMAPLE 14: [Ru₂(OAc)₄]Cl

Available nitric oxide was reduced by 47% at 100µM.

EXAMPLE 15: [Ru(NH₃)₅Cl]Cl₂

Available nitric oxide was reduced by 86% at 100µM test compound.

20

EXAMPLE 26: [Ru(maltolato)₃]

Available nitric oxide was reduced by 71% at 100µM.

- 34 -

TABLE 1

		% Decrease of Available Nitric Oxide	
5	Example 1	25µM	12
		50µM	23
		100µM	75
10	Example 2	100µM	82
	Example 3	100µM	42
	Example 6	100µM	77
15	Example 14	100µM	47
	Example 15	100µM	86
	Example 26	100µM	71

15 EX-VIVO TESTS

EXAMPLE 2

Application of test compound resulted in a dose-dependent
20 vasoconstriction at 10µM and 100µM. This effect was reversible by washout with
Krebs solution.

- 35 -

EXAMPLE 3

Application of test compound resulted in a dose-dependent vasoconstriction at 10 μ M and 100 μ M. This effect was reversible by washout with
5 Krebs solution.

EXAMPLE 14

Application of test compound resulted in a dose-dependent vasoconstriction at 10 μ M and 100 μ M. This effect was reversible by washout with
10 Krebs solution.

EXAMPLE 15

15 Application of test compound resulted in a dose-dependent vasoconstriction at 10 μ M and 100 μ M. This effect was reversible by washout with
Krebs solution.

EXAMPLE 26

20 Application of test compound resulted in a dose-dependent vasoconstriction at 10 μ M and 100 μ M and 1000 μ M. This effect was reversible by
washout with Krebs solution.

- 36 -

TABLE 2

% Vasoconstriction

	Example 2	10 μ M	20
		100 μ M	69
5	Example 3	10 μ M	17
		100 μ M	59
	Example 14	10 μ M	11
		100 μ M	40
	Example 15	10 μ M	16
10		100 μ M	86
	Example 26	10 μ M	10
		100 μ M	18
		1000 μ M	25

CLAIMS

1. The use of a neutral, anionic or cationic metal complex having at least one site for coordination with NO, of formula

5



in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease,

10

where: M is a metal ion or a mixture of metal ions;

X is a cation or a mixture of cations;

L is a ligand, or mixture of ligands each containing at least two different donor atoms selected from the elements of Group IV,

15 Group V or Group VI of the Periodic Table;

Y is a ligand, or a mixture of the same or different ligands each containing at least one donor atom or more than one donor atom, which donor atom is selected from the elements of Group IV, Group V or Group VI of the Periodic Table;

20 and Z is a halide or pseudohalide ion or a mixture of halide ions and pseudohalide ions;

a = 1-3; b = 0-12; c = 0-18; d = 0-18; e = 0-18; and n = 0-10;

provided that at least one of c, d and e is 1 or more;

- 38 -

and where c is 0; b is also 0;

and where a is 1; c, d and e are not greater than 9;

and where a is 2; c, d and e are not greater than 12.

5 2. The use of a complex of formula I in the manufacture of a medicament for the treatment of NO-overproduction related disease.

10 3. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 1, wherein M is a first, second or third row transition metal ion.

4. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 1, wherein M is an Rh, Ru, Os, Mn, Co, Cr or Re ion.

15

5. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 4, wherein M is an Rh, Ru or Os ion.

20 6. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 5, wherein M is in oxidation state III.

- 39 -

7. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to any of claims 1-6, wherein X is a mono-, di- or tri-valent cation.
- 5 8. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 7, wherein X is H⁺, K⁺, Na⁺, NH₄⁺ or Ca²⁺.
9. The use of a complex of formula I in the manufacture of a
10 medicament for the attenuation of NO levels where NO is implicated in disease, according to any of claims 1-8, wherein L is a ligand containing both nitrogen and oxygen donor atoms.
10. The use of a complex of formula I in the manufacture of a
15 medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 9, wherein L is edda, edta, nta, dipic, pic, dtpa, hedtra, tedita or dtedita.
11. The use of a complex of formula I in the manufacture of a
20 medicament for the attenuation of NO levels where NO is implicated in disease, according to any of claims 1-10, wherein Y is a ligand containing at least one of N, O, S, C or P donor groups.

- 40 -

12. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 11, wherein said N donor group is ammine, amine, amide, nitrile or nitride or derivatives thereof.

5

13. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 11, wherein said O donor group is carboxylic acid, ester or derivatives thereof, water, oxide, sulphoxide, hydroxide, acetate, lactate, propionate, oxalate or maltolate.

10

14. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 11, wherein said S donor group is sulphoxide, dialkylsulphide, dialkylcarbamate, dithiocarbamate, or dithiophosphate.

15

20

15. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 11, wherein said C donor group is carbon monoxide or isocyanide.

20

16. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 11, wherein said P donor group is trialkylphosphine.

- 41 -

17. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to any preceding claim, wherein Z is a halide.
- 5 18. The use of a complex of formula I in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease, according to claim 17, wherein Z is chloride.
19. The use of an optionally hydrated ruthenium complex of formula
- 10
- [Ru(H_{0.6}L'')_{1.3}Y_{0.2}Cl_{1.4}]^{(0-4)±} formula II,
- where L'' is an amide or ester or derivative thereof, or a polydentate aminocarboxylate ligand, for example edda, tropolone edta, nta, dipic, pic, dtpa, hedra, tedta or dtedta or a mixture of any of these, and Y is as defined above and may for example be selected from acetylacetone (acac), a β-diketonate, water, dimethylsulphoxide (dmso), carboxylate, bidentate carboxylate, catechol, kojic acid, maltol, hydroxide, tropolone, malonic acid, oxalic acid, 2,3-dihydroxynaphthalene, squaric acid, acetate, a sulphate and a glycolate in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease.

- 42 -

20. A pharmaceutical composition containing an optionally hydrated complex of formula



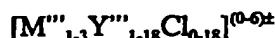
formula III

5

wherein Y is a sulphur donor ligand.

21. The use of an optionally hydrated complex of ruthenium of formula

10



formula III

15

where M''' is ruthenium and Y''' is an oxygen-donor ligand such as acetate, lactate, water, oxide, propionate, oxalate or maltolate or a combination of these, in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease.

22. The use of an optionally hydrated complex of ruthenium of formula

20



formula IV

where Y^{IV} is a nitrogen-donor ligand such as ammine, ethylenediamine (en), pyridine (py), 1,10-phenanthroline (phen), 2,2'-bipyridine (bipy), 1,4,8,11-tetraaza-cyclotetradecane (cyclam), 2,3,7,8,12,13,17,18-octaethylporphyrin (oep) or a

- 43 -

combination of these, in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease.

23. The use of an optionally hydrated complex of ruthenium or osmium
5 of general formula



where Y is a combination according to claims 19, 20, 21, or 22 in the manufacture
10 of a medicament for the attenuation of NO levels where NO is implicated in disease.

24. The use of an optionally hydrated complex of general formula
15 $[M_{1-3}Y_{1-18}Cl_{0-18}]^{(0-6)\pm}$

where Y is a sulphur donor ligand, in the manufacture of a medicament for the attenuation of NO levels where NO is implicated in disease.

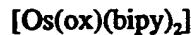
- 20 25. A pharmaceutical composition containing an optionally hydrated ruthenium complex as described in claim 19.

- 44 -

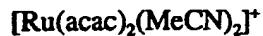
26. A pharmaceutical composition containing an optionally hydrated complex of ruthenium as described in claim 21, wherein Y^{III} is an oxygen donor ligand selected from the group acetate, lactate, oxide, propionate and maltolate.

5 27. A pharmaceutical composition containing an optionally hydrated complex of ruthenium as described in claim 22, wherein Y^{IV} is a nitrogen donor ligand selected from the group en, py, phen, bipy, cyclam or oep.

10 28. A pharmaceutical composition containing an optionally hydrated complex of formula



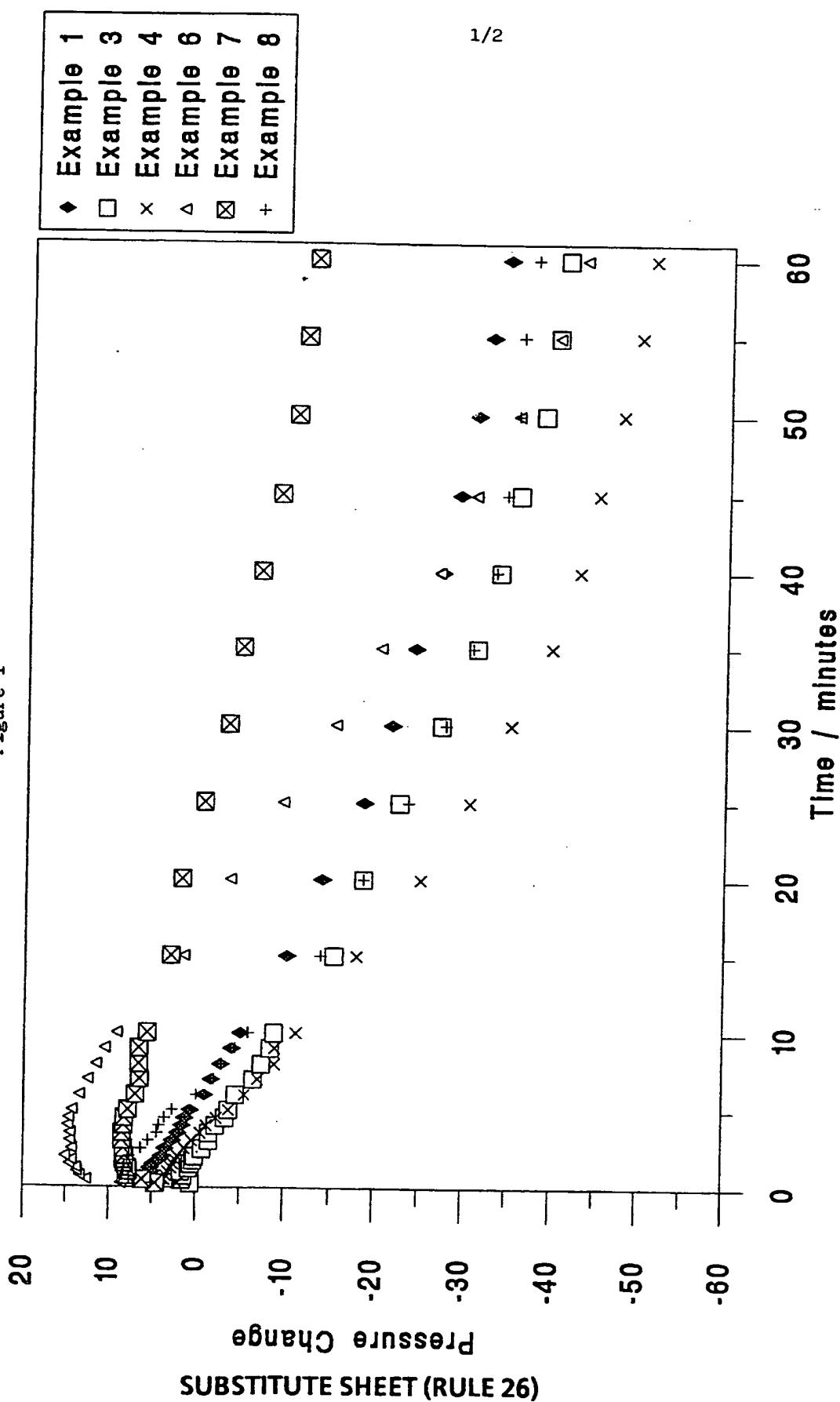
15 29. A pharmaceutical composition containing an optionally hydrated complex of formula



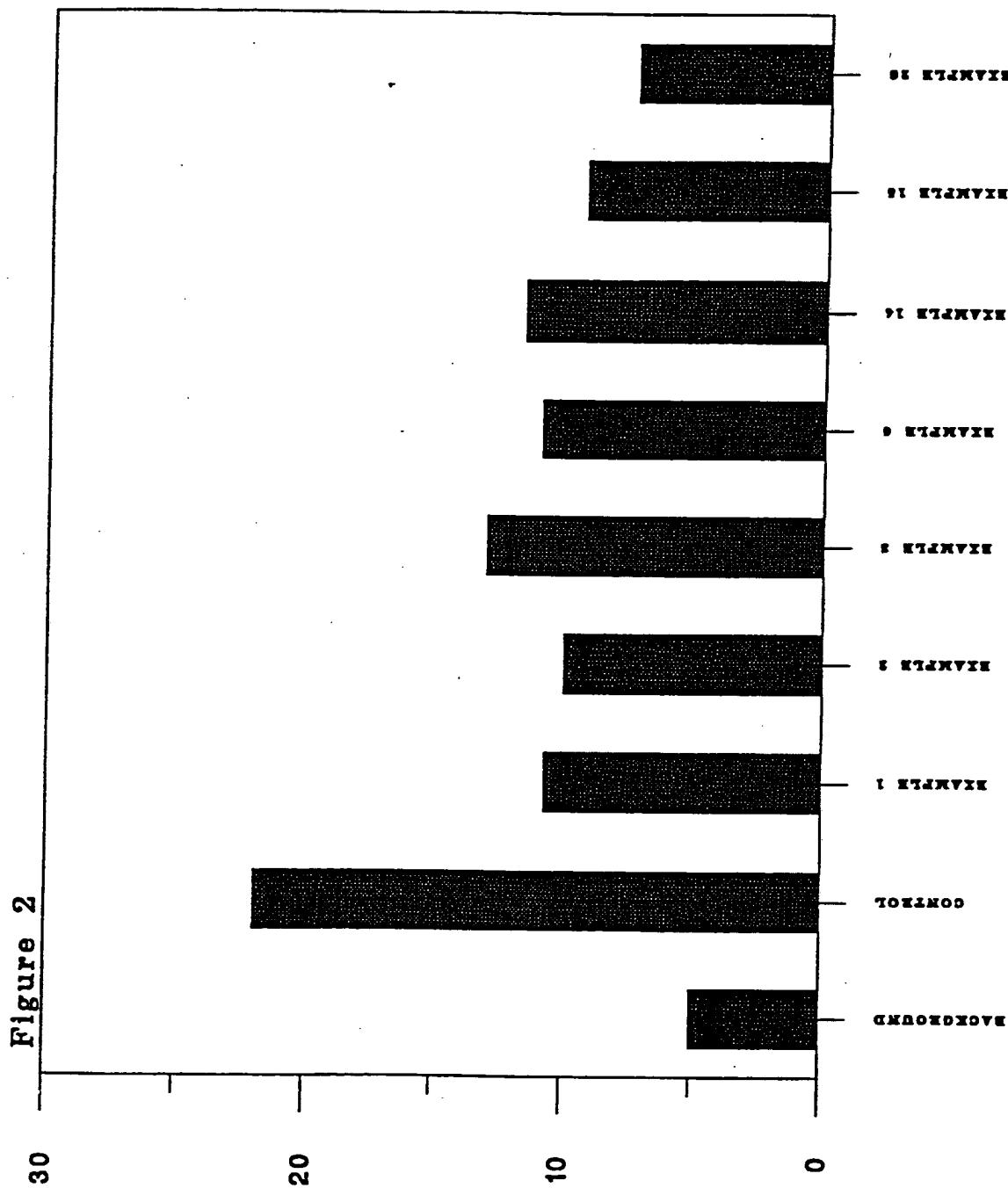
20

1/2

Figure 1



2/2



AVAILABLE NITRIC OXIDE (micromoles/l)

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

Int'l. Application No

PCT/GB 94/01817

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 A61K31/28 A61K33/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,90 13553 (BOEHRINGER BIOCHEMIA ROBIN) 15 November 1990	25
A	see abstract; claims; examples ---	27
X	WO,A,86 00804 (BYK GULDEN LOMBERG) 13 February 1986	27
	see abstract; claims; examples ---	
X	WO,A,86 00905 (BYK GULDEN LOMBERG) 13 February 1986	27
	see abstract; claims; examples ---	
X	US,A,5 157 032 (BARTON) 20 October 1992 see abstract see column 6, line 4 - line 12 see column 9, line 48 - column 12, line 38 see column 27, line 28 - line 41; claims; tables 13-14 ---	27
	-/-	

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

25 October 1994

11.11.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentiaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+31-70) 340-3016

Authorized officer

Hoff, P

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/GB 94/01817

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER RESEARCH, vol.37, 1977 pages 2662 - 2666 T. GIRALDI ET AL. 'ANTITUMOR ACTION OF TWO RHODIUM AND RUTHENIUM COMPLEXES IN COMPARISON WITH CIS-DIAMMINEDICHLOROPLATINUM(II)' see the whole document ---	20
X	FR,A,1 482 811 (THE AUSTRALIAN NATIONAL UNIVERSITY) 2 June 1967 see the whole document ---	27
A	AUSTRALIAN JOURNAL OF CHEMISTRY, vol.17, 1964 pages 325 - 336 D.A. BUCKINGHAM ET AL. 'MONO- AND BIS-(2,2'-BIPYRIDINE) AND (1,10-PHENANTHROLINE) CHELATES OF RUTHENIUM AND OSMIUM' cited in the application see the whole document ---	28
A	CHEMISTRY LETTERS, no.3, 1990 pages 381 - 384 Y. KASAHARA ET AL. 'REACTION OF TRIS (beta-DIKETONATO)RUTHENIUM(III) COMPLEXES WITH STRONG ACIDS IN ACETONITRILE' cited in the application see the whole document ---	29
A	INORGANIC CHEMISTRY, vol.30, no.4, 1991 pages 629 - 635 M.R. RHODES ET AL. 'ELECTROCATALYTIC REDUCTION OF NITRITE ION BY EDTA COMPLEXES OF IRON(II) AND RUTHENIUM(II)' see the whole document ---	1-29
A	INORGANIC CHEMISTRY, vol.23, no.16, 1984 pages 2466 - 2472 D.W. PIPES ET AL. 'COMPARISON BETWEEN POLYPYRIDYL NITROSYL COMPLEXES OF OSMIUM(II) AND RUTHENIUM(II)' ---	1-29
		-/-

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 94/01817

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THE PHYSIOLOGICAL SOCIETY, vol.467, January 1993 page 165P T.D. CARTER ET AL. 'PHOTOCHEMICAL RELEASE OF NITRIC OXIDE FROM RUTHENIUM NITROSYL TRICHLORIDE: RELAXATION OF RABBIT ISOLATED AORTIC RINGS MEDIATED BY PHOTO-RELEASE OF NITRIC OXIDE' see the whole document -----	1-29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB94/01817

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: 1-27 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
FOR FURTHER INFORMATION SEE ANNEX

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

CONTINUATION OF BOX I.2

In view of the large number of compounds which are defined by the general formulas of claims 1, 19-24, the search was limited to the inventive part of the molecule and to the compounds mentioned in the examples.
(PCT: Art.6; Guidelines.....Part B, Chapt.II.7 last sentence and Chapt. III, 3.7)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l. Application No

PCT/GB 94/01817

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9013553	15-11-90	AU-B-	629341	01-10-92
		AU-A-	5530290	29-11-90
		EP-A-	0471709	26-02-92
-----	-----	-----	-----	-----
WO-A-8600804	13-02-86	EP-A-	0188585	30-07-86
-----	-----	-----	-----	-----
WO-A-8600905	13-02-86	AU-B-	570826	24-03-88
		AU-A-	4804385	25-02-86
		EP-A,B	0191096	20-08-86
		US-A-	4843069	27-06-89
		JP-T-	61502761	27-11-86
-----	-----	-----	-----	-----
US-A-5157032	20-10-92	US-A-	4721669	26-01-88
		AU-A-	5352086	13-08-86
		EP-A-	0211043	25-02-87
		US-A-	4980473	25-12-90
		WO-A-	8604244	31-07-86
		US-A-	5112974	12-05-92
		US-A-	5225556	06-07-93
-----	-----	-----	-----	-----
FR-A-1482811		CH-A-	415657	
		CH-A-	447190	
		GB-A-	956242	
		NL-A-	260509	
		NL-A-	260510	
-----	-----	-----	-----	-----